

# NATURAL HYBRIDIZATION AMONG THREE SYMPATRIC *BAPTISIA* (FABACEAE) SPECIES IN NORTH CENTRAL TEXAS

MATTHEW A. KOSNIK,<sup>1</sup> GEORGE M. DIGGS, JR.  
and PEGGY A. REDSHAW

*Department of Biology*  
*Austin College*  
*Sherman, TX 75090-4440 U.S.A.*

BARNEY L. LIPSCOMB  
*Botanical Research Institute of Texas*  
*509 Pecan Street*  
*Fort Worth, TX 76102-4060 U.S.A.*

## ABSTRACT

A north central Texas *Baptisia* (Fabaceae, Faboideae, Thermopsidae) hybrid complex containing three parents (*B. australis*, *B. sphaerocarpa*, and *B. bracteata*) and the three F<sub>1</sub> hybrids is investigated using morphological, spectrophotometric, and isozyme electrophoretic methods. Two of the three hybrids (*B. × bushii* and *B. × bicolor*) have been previously named, but the *B. australis* × *B. sphaerocarpa* hybrid (*B. × variicolor*) is here described for the first time. This is the first report of sympatric occurrence of these three *Baptisia* species and all the possible F<sub>1</sub> hybrids. Morphological evidence for introgressive hybridization involving *B. australis* and *B. sphaerocarpa* is presented.

## RESUMEN

Un complejo de híbridos del norte de Texas, *Baptisia* (Fabaceae, Faboideae, Thermopsidae) que contiene tres padres (*B. australis*, *B. sphaerocarpa*, y *B. bracteata*) y tres híbridos F<sub>1</sub> se investiga por métodos morfológicos, espectrofotométricos, y de electroforesis de isozimas. Dos de los tres híbridos (*B. × bushii* y *B. × bicolor*) habían sido nombrados previamente, pero el híbrido de *B. australis* × *B. sphaerocarpa* (*B. × variicolor*) se describe aquí como nuevo. Esta es la primera cita de la existencia simpátrica de estas tres especies *Baptisia* y todos los híbridos posibles de F<sub>1</sub>. Se presenta la evidencia morfológica de hibridación introgresiva que afecta *B. australis* y *B. sphaerocarpa*.

## INTRODUCTION

The genus *Baptisia*, commonly called Wild Indigo, is a perennial, herbaceous, and conspicuously flowered legume genus with about 25–30 species occurring in the eastern United States. In Texas they occur in remnant native habitats and are common weeds in overgrazed pastures (Turner 1959).

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<sup>1</sup>Present address: Department of Geophysical Sciences, University of Chicago, 5734 South Ellis Avenue, Chicago, IL 60637, makosnik@midway.edu.

Taxonomic difficulties have been common in the group due to extensive hybridization and introgression among species (Correll & Johnston 1970). This taxonomic confusion and the proliferation of secondary compounds within the genus led to early use of chemical taxonomic methods (e.g. chromatography by Turner & Alston 1959; Alston & Turner 1962; Alston & Turner 1963), and later detailed investigation of flavonoid distributions within the genus (Markham et al. 1970; Dement & Mabry 1975). Because of this work, a great deal is known about *Baptisia* both morphologically and chemically.

Three *Baptisia* species occur in the area of north central Texas studied, *B. australis* (L.) R. Br. ex W.T. Aiton var. *minor* (Lehm.) Fern. (a blue-violet-flowered species, Fig. 1), *B. sphaerocarpa* Nutt. (a yellow-flowered species, Fig. 1), and *B. bracteata* Muhl. ex Ell. var. *leucophaea* (Nutt.) Kartesz & Gandhi (a cream-flowered species, Fig. 1). Nomenclature follows Kartesz (1994). *Baptisia australis* reaches the southern extreme of its range in the north central Texas area, *B. bracteata* reaches its western boundary in the area, and *B. sphaerocarpa* reaches its northern extreme in central Oklahoma (Larisey 1940a). The area where all three ranges overlap is limited to north central Texas, southern Oklahoma, and western Arkansas (Fig. 2).

Hybridization and multispecies hybrid complexes are well documented in the genus *Baptisia*, both through traditional morphological methods and through extensive chemical analysis. In her monograph on the genus *Baptisia*, Larisey (1940a) recognized eight *Baptisia* hybrids and cited the relatively frequent occurrence of hybridism, particularly in areas where the borders of the geographically larger species overlap. At several north central Texas locations in Grayson County and neighboring Fannin County, several *Baptisia* species coexist and have been suspected of hybridizing (Kosnik 1996). The *B. australis*  $\times$  *B. bracteata* hybrid, with an intermediate or light blue-violet flower (Fig. 1), has been described previously (*B.  $\times$  bicolor* Greenm. & Larisey), and the *B. bracteata*  $\times$  *B. sphaerocarpa* hybrid, with an intermediate or light yellow flower (Fig. 1), has also been described previously (*B.  $\times$  bushii* Small). However, the proposed *B. australis*  $\times$  *B. sphaerocarpa* hybrid, with a flower color varying from brick-red and yellow to orangish to bluish-violet and yellow (Figs. 1 and 3), has not been previously described in the literature.

*Baptisia bracteata*, *B. australis*, and *B. sphaerocarpa* have also each independently been observed hybridizing with other members of the genus. *Baptisia australis*  $\times$  *B. bracteata* hybrids have been observed in Kansas (Hitchcock 1894). *Baptisia sphaerocarpa*  $\times$  *B. laevicaulis* hybrids have been described using morphological and chemical methods in southeastern Texas by Turner and Alston (1959) and Alston and Turner (1962). *Baptisia sphaerocarpa*  $\times$  *B. leucantha* hybrids were observed in Oklahoma by Engelmann (1878),

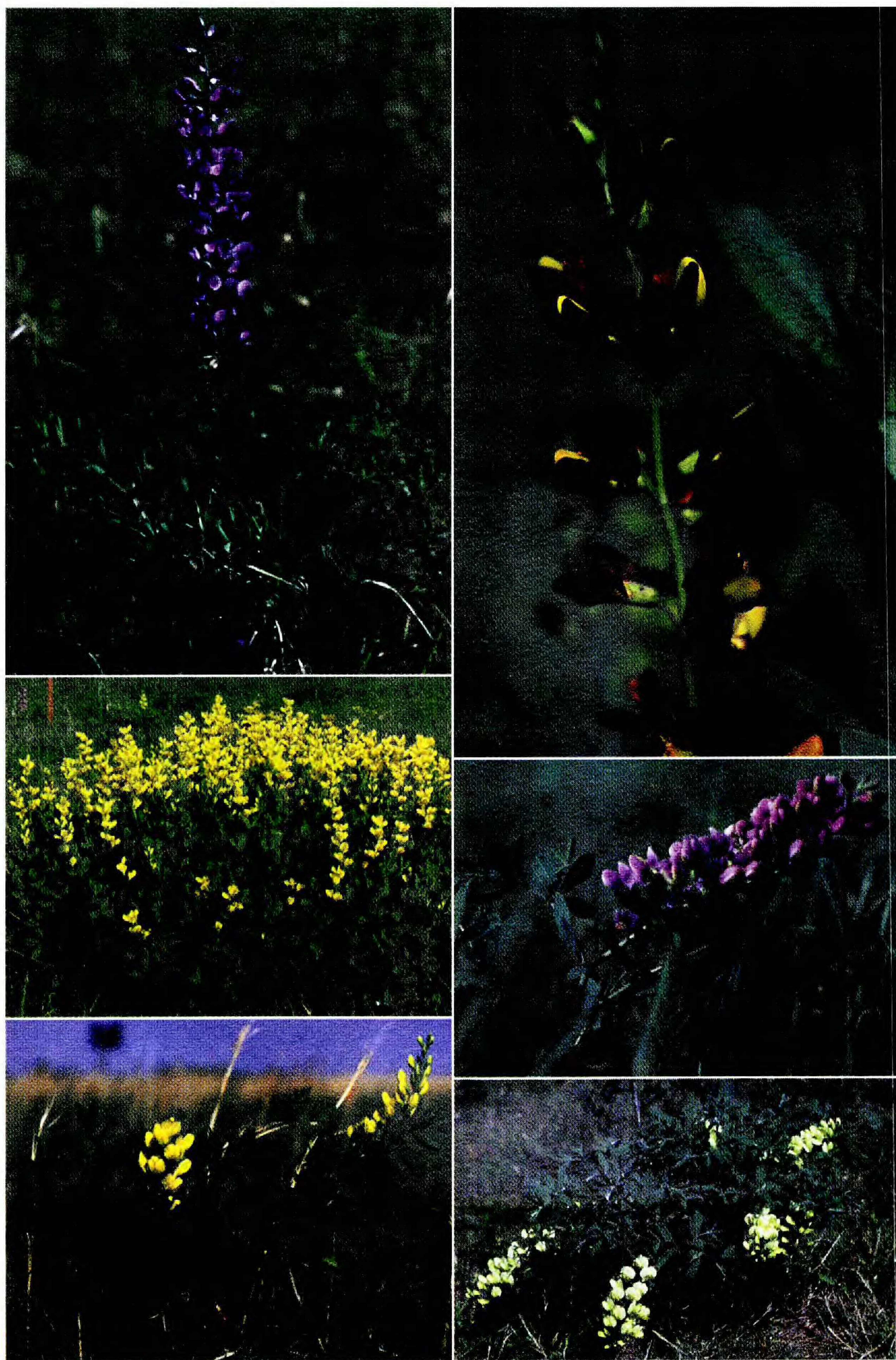


FIG. 1. Photographs of parental species and hybrids. Clockwise from upper left: *Baptisia australis*, *B. × variicolor*, *B. × bicolor*, *B. bracteata*, *B. × bushii*, and *B. sphaerocarpa*.

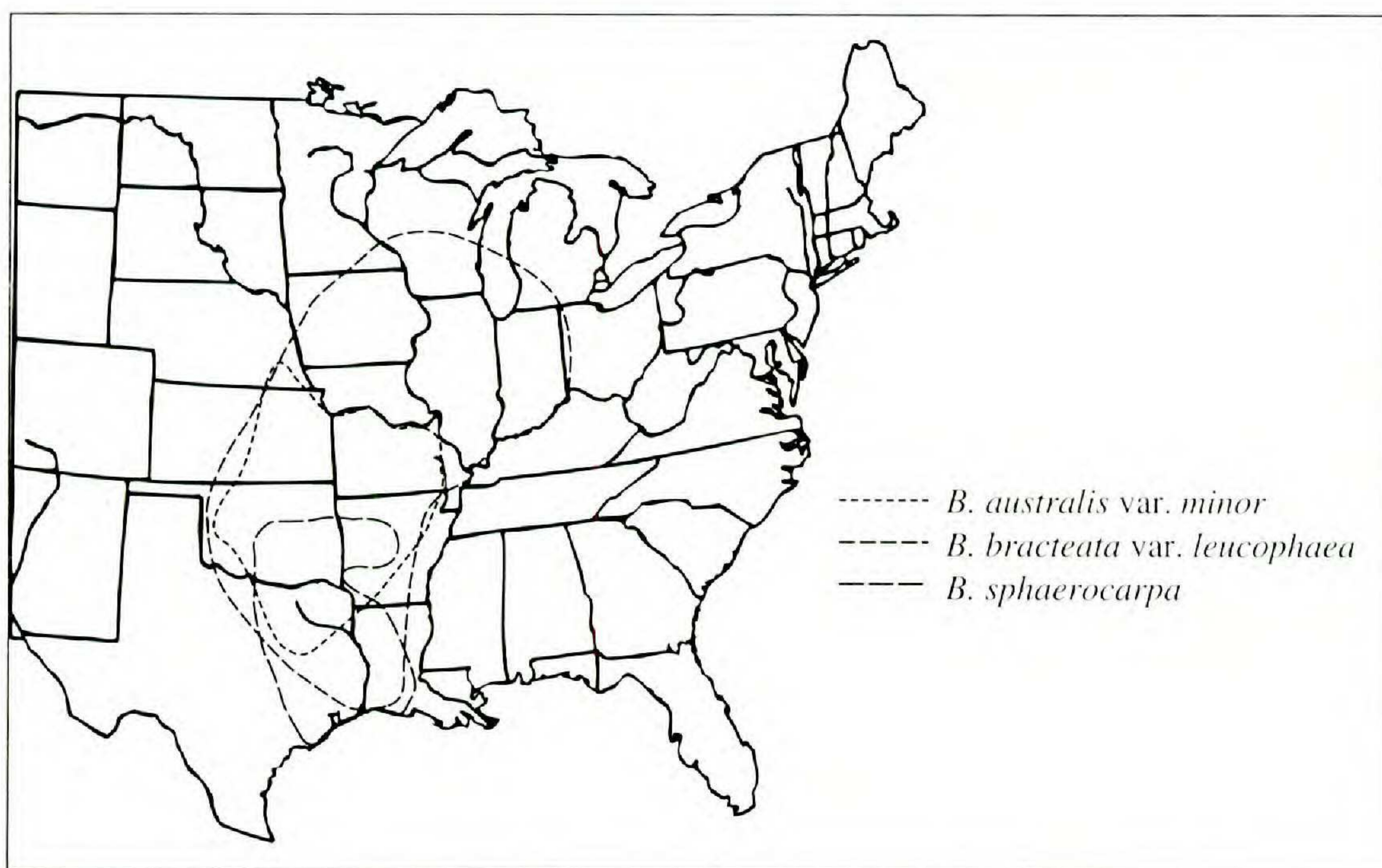


FIG. 2. Distribution map for the parental species. (Modified from Larisey 1940a).

and in southeastern Texas they were described morphologically by Larisey (1940b) and morphologically and chemically by Alston and Turner (1962). *Baptisia sphaerocarpa*  $\times$  *B. bracteata* hybrids were observed in Louisiana by Harper (1938). However, it is only in north central Texas that all three of these species and all three possible  $F_1$  hybrids have been observed in mixed populations. Introgressive hybridization (Anderson 1949), or the movement of genes from one species into another via fertile hybrids, has also been suggested for *Baptisia* (e.g., Alston & Turner 1963; Correll & Johnson 1970).

#### METHODS

*Collection localities.*—Plant material was collected at 11 sites in Grayson and Fannin counties, Texas. At three additional sites hybrids were observed but not collected. Table 1 summarizes collections and observations. Coordinates for each site (Table 1) were determined using a Garmin 45 Global Positioning System. Voucher specimens are deposited at the Botanical Research Institute of Texas (BRIT) in Fort Worth.

*Morphological Measurements.*—Measurements of a number of characters were made in the field, prior to collection and pressing. Vegetative height was measured from the ground to the highest leaves (0.5 cm increments). Plant height was measured from the ground to the maximum height (0.5 cm increments). Raceme angle was measured with a weighted protractor (2.5° increments, 0° considered vertical and 90 considered horizontal). Total flower length (from the base of the flower to the tip of the keel or wings), total

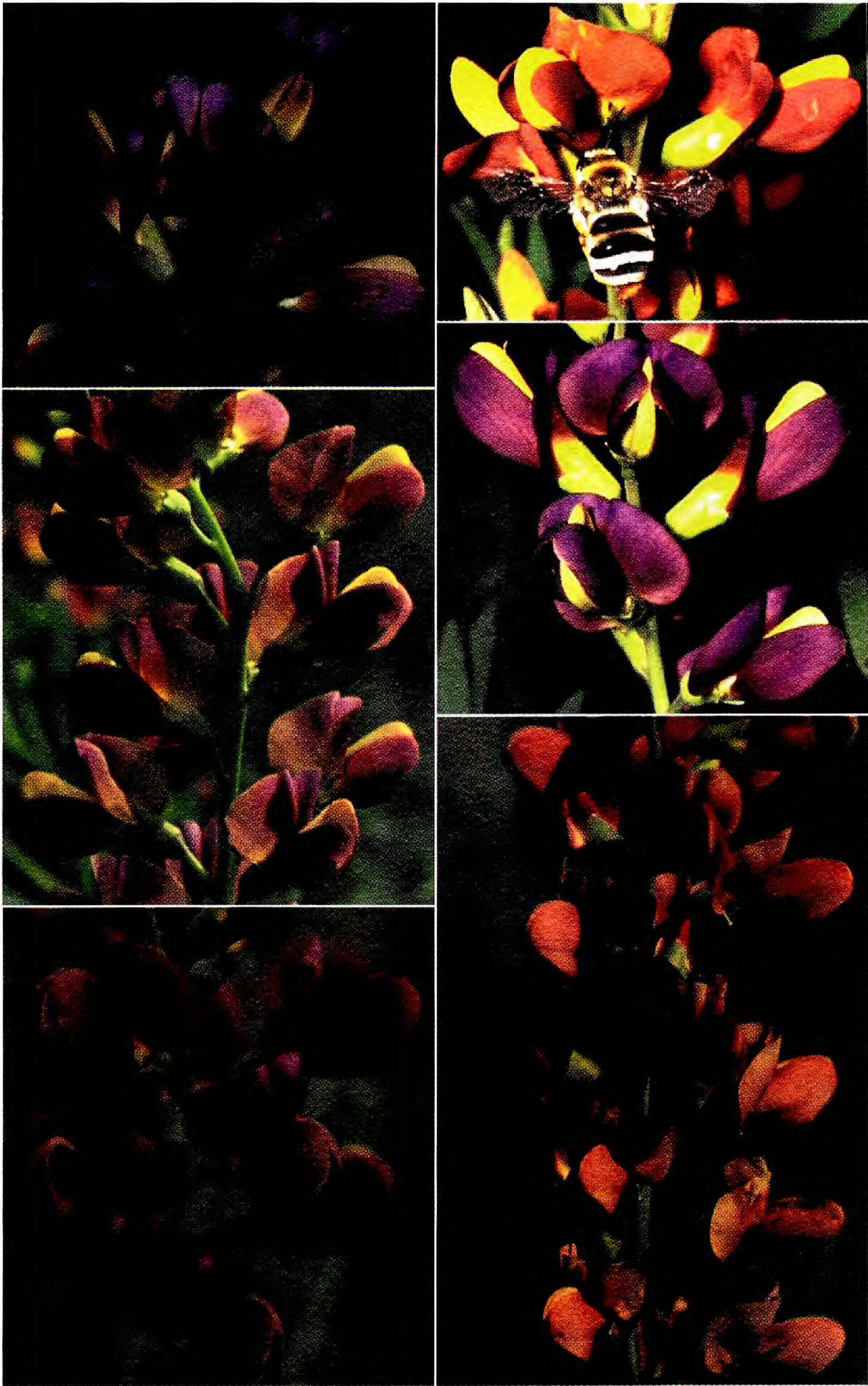


FIG. 3. Photographs of *B. × variicolor* (*B. australis* × *B. sphaerocarpa*). Note variable flower color.

TABLE 1. Sites and collections. Plants are indicated: B = *B. australis*, BY = *B. × variicolor*, Y = *B. sphaerocarpa*, YC = *B. × bushii* (*sphaerocarpa* × *bracteata*), C = *B. bracteata*, CB = *B. × bicolor* (*bracteata* × *australis*). Collection number indicates the number of herbarium specimens collected at each location, an X indicates that the plant was observed but not collected for pressing.

Location Name	Position		Collection/Observation					
	Latitude	Longitude	B	BY	Y	YC	C	CB
FAN-A6	33° 31'	96° 20'	3	5	10	4	10	7
G36-CY	33° 44'	96° 49'			10	3	10	
GEO-B	33° 36'	96° 31'	10					
HAG-C	33° 45'	96° 48'					5	
MEM-CY	33° 46'	96° 38'			5	5	5	
PRE-A6	33° 48'	96° 38'	6	2	X	2	2	7
PRE-BY	33° 47'	96° 36'	7	1	2			
HAG-Y	33° 45'	96° 48'			10			
1417-Y	33° 41'	96° 39'			5			
PWR-B	33° 31'	96° 28'	10					
POT-A6	33° 47'	96° 38'	X					1
ORIGIN	33° 32'	96° 22'		X	X			
B36-CY	33° 44'	96° 48'			X	X	X	
FAN-CY	33° 31'	96° 20'			X	X	X	
Total			36	8	42	14	32	15

flower height (from the bottom of the keel or wings to the tip of the banner), maximum horizontal width of the wings, and maximum banner width were measured with digital calipers (0.01 mm increments). All flower measurements were made on the lowest fully developed, intact flower on the best developed inflorescence, with at least one fully developed flower above it.

Measurements of all other vegetative characters were made after pressing using digital calipers (0.01 mm increments). These included pedicel length (of the measured flower); floral bract length, maximum width, width at attachment; petiole length; central leaflet length, maximum width, distance to the maximum width (from the leaflet base); and stipule length, maximum width, width at attachment, and distance to maximum width (from the stipule base). The leaflet measurements were taken on the center leaflet of the first undamaged, well developed leaf below the raceme. Stipule measurements were taken from the same leaf, unless the leaf did not have a stipule, in which case the stipule closest to that leaf was measured (this was often the case with *B. sphaerocarpa*).

Fruit characters were chosen to examine for evidence of introgressive

hybridization between *B. australis* and *B. sphaerocarpa* because of the very different fruit shapes and sizes of these taxa and thus the increased probability of finding unambiguous evidence of introgression. Individuals from the hybrid population were labeled as to flower color during the spring flowering season and fruits were subsequently collected when mature. Fruit measurements were taken using digital calipers (0.01 mm increments) from completely dried fruits collected in the field in June and July from FAN-A6, GEO-B, HAG-Y, and PWR-B (Table 1). These included maximum fruit length (not counting stipe or beak), height, width, and length of beak (from where fruit body narrows dramatically apically to tip of beak). Measurements were taken on the lowest well developed, well preserved fruit on an infructescence.

The Royal Horticultural Society Colour Chart (1966) was also used in an attempt to standardize color measurements. The lowest, mature, undamaged flower on the raceme was color matched before it dried.

*Statistical Analyses.*—Morphological measurements were analyzed using principal component analysis, multiple discriminant analysis (a multivariate statistical technique sometimes called canonical analysis), and analysis of variance and run with Minitab 10.5Xtra (1995) and BioStat II (Pimentel 1995) on a Macintosh Centris 650. Because missing data are not allowed in these analyses, only the characters listed in Tables 2 and 3 were used. Principal component analysis attempts to unravel patterns of variation within the entire dataset, while multiple discriminant analysis attempts to minimize within group variation while maximizing between group variation. Discriminant analysis computes synthetic characters by weighting dataset characters to maximally separate the groups. For the discriminant analysis individuals were assigned to one of six groups based on flower color.

*Spectrophotometric Analysis.*—Flowers from four to nine individuals of each of the six taxa were examined spectrophotometrically. Flowers from three sites (FAN-A6, MEM-CY, and PRE-A6) were selected to represent the maximum variation seen within the populations. Petals from a fresh, undamaged, mature flower were ground completely in 2 ml of a 1:4, 1% HCl: 100% EtOH solution; the samples were then centrifuged for three minutes to separate large particulate material. One ml of the resulting extract was diluted with an additional 2.5 ml of solvent. An ultraviolet-visible light absorbance spectrum was then measured using a Beckman DU-70 scanning spectrophotometer; absorbance scans were run at 1200 nm/min. from 200 nm to 700 nm. Absorbances for peaks at 422 nm, 449 nm, and 540 nm ( $\pm 2$  nm) were recorded.

*Enzyme Electrophoretic Analysis.*—Leaf samples were obtained from 21 individuals including *B. australis*, *B. sphaerocarpa*, and all three F<sub>1</sub> hybrids at FAN-A6 in July 1990. *Baptisia bracteata* was not examined. Starch gel

enzyme electrophoretic procedures generally followed Soltis et al. (1983). The tris-HCl grinding buffer-PVP solution described by Soltis et al. (1983) was used with a PVP 40,000 concentration of 12% (wt/vol). Starch gel concentration was 12.5%.

The following enzymes were resolved: aspartate aminotransferase (AAT or GOT), glyceraldehyde 3-phosphate dehydrogenase (G3PDH), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6PGD), shikimate dehydrogenase (SKDH), and triosephosphate isomerase (TPI). Buffer systems follow Soltis et al. (1983): G3PDH and SKDH were resolved on a modification of gel and electrode buffer 1; GOT and TPI on a modification of buffer 8 (modification described by Soltis & Soltis 1987); 6PGD on a modification of buffer 9; and MDH and IDH on a morpholine system (electrode buffer: 0.04 M citric acid, pH 7.5 by addition of N-(3-aminopropyl)-morpholine; gel buffer: dilute electrode buffer 1:25). Staining procedures followed Soltis et al. (1983).

## RESULTS

*Morphological Analysis.*—Based simply on flower color, the three parents and their intermediate putative hybrids are easily distinguished. A variety of morphological characters also suggest hybridization. Simple two character plots of raceme angle versus flower size (height) or leaflet size (length) distinguish six basic morphological groups which support the hybridization hypothesis (Fig. 4). The three hybrids are generally intermediate morphologically between the parents. These graphs also show considerable overlap among hybrid taxa.

Principal component analysis separates the taxa in a manner similar to the two character plots. The plot of the first principal component versus the second principal component illustrates that variation within all six taxa (3 parental species and 3 hybrids) is less than the variation between taxa (Fig. 5)—in other words, groups can be observed though there is some overlap. Individuals of the three parental species form discernable clusters toward the periphery of the plot, while all the suspected hybrid taxa are generally intermediate between the purported parental species. Character weightings are given in Table 2.

Discriminant analysis separates the taxa with greater resolution than the principal component analysis, but still does not completely separate all individuals of all the taxa (Fig. 6). The discriminant analysis supports 94% of the original flower color grouping. The 6% of the individuals which are ambiguously grouped by the discriminant analysis on the basis of the morphological data are clearly distinct in other characters, such as flower color. The linear discriminant functions are given in Table 3.

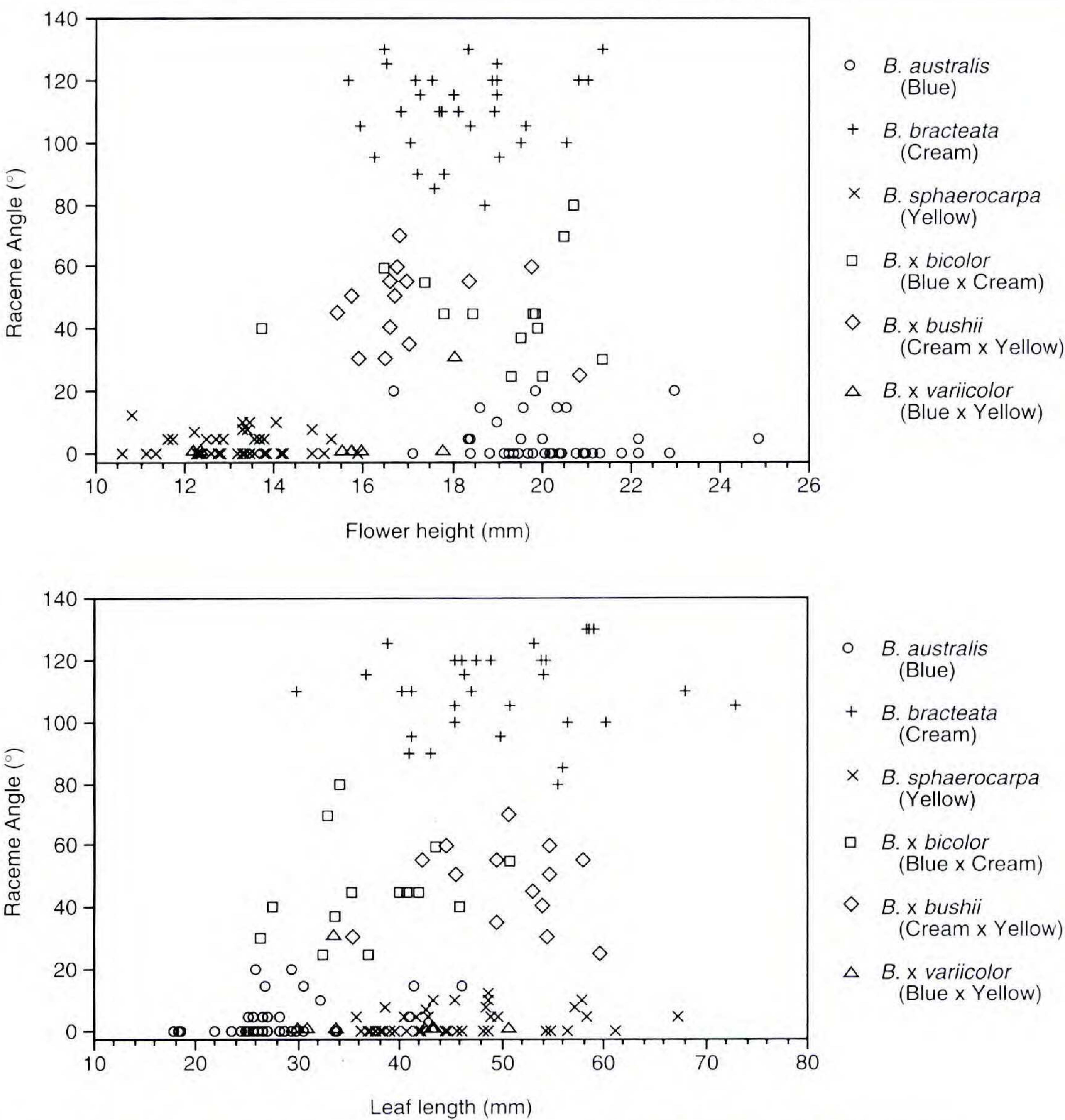


FIG. 4. Two character plots of raceme angle versus flower height and leaf length.

There are additional characters, suggestive of hybridization, that were not included in the principal component analysis or the discriminate analysis because they could not be accurately and consistently quantified. These include hairs (too numerous to count effectively on *B. bracteata*), flower bracts (length, maximum width, and distance to maximum width cannot be measured on plants without persistent bracts), and flower color (not continuously quantifiable). *Baptisia bracteata* is densely pubescent, has large persistent floral bracts (10–30 mm long), and cream flowers, while *B. sphaerocarpa* and *B. australis* are glabrous, have smaller, early deciduous bracts (0–5 mm long), and have vividly colored flowers (bright yellow in the case of *B. sphaerocarpa* or blue-violet in the case of *B. australis*). The hybrids, *B. x bushii* (*B. sphaerocarpa* × *B. bracteata*) and *B. x bicolor* (*B. australis* × *B. bracteata*) show intermediate values for these characters.

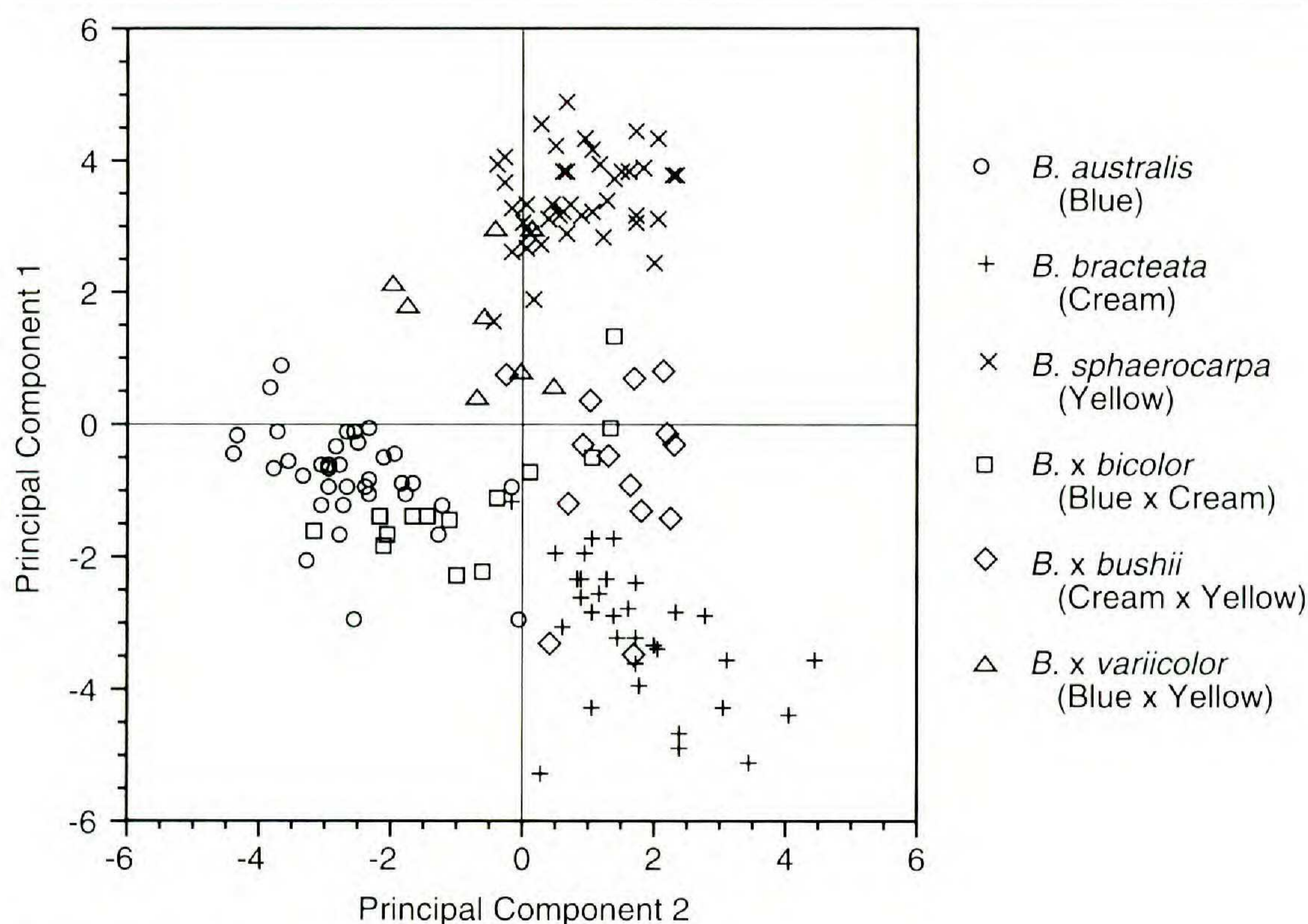


FIG. 5. Principal components analysis.

*bracteata*), are intermediate between their respective parents in all of these characters. *Baptisia sphaerocarpa* and *B. australis* are easily distinguished by flower color and plant form. *Baptisia sphaerocarpa* has numerous flexible, vegetative branches creating a bushy form, commonly multiple racemes per plant, and intense yellow flowers. *Baptisia australis* has fewer, much more rigid branches, typically only one raceme per plant, and a blue-violet flower color that varies greatly in saturation between plants. *Baptisia variicolor* is generally vegetatively intermediate, but often is superficially more similar to *B. sphaerocarpa*; they have vegetative branches creating a bushy form and often have multiple racemes per plant. The flower color of *B. × variicolor* is strikingly variable. It ranges from intermediate to a patch-work combination of the parental flower colors, with different hybrid individuals having flowers ranging from brick-red and yellow to orangish to bluish-violet and yellow. This variation can be seen in Figs. 1 and 3. The Royal Horticultural Society Colour Chart measurements are an indication of the variation in color (Table 4).

*Spectrophotometric Analysis.*—The absorbance spectra indicate three diagnostic wavelengths (Fig. 7). *Baptisia australis* has a large peak at 540 nm, *B. sphaerocarpa* has distinct peaks at 422 nm and 449 nm, and *B. bracteata* has low absorbance at all of the diagnostic wavelengths. *Baptisia variicolor* clearly shows both parental peaks, while *B. × bicolor* and *B. × bushii* have peak

TABLE 2. Principal component character weightings.

Variable	Component 1	Component 2
Plant total height	0.272	-0.205
Plant vegetative height	0.275	-0.063
Raceme angle	-0.276	0.264
Flower total length	-0.236	-0.326
Flower max. wing width	-0.036	0.123
Flower total height	-0.291	-0.265
Flower max. banner width	-0.257	-0.300
Petiole length	-0.284	0.184
Pedicle length	0.108	-0.046
Leaflet length	-0.002	0.479
Leaflet max. width	0.198	0.344
Leaflet distance to max. width	-0.042	0.438
Stipule length	-0.332	0.114
Stipule max. width	-0.346	0.096
Stipule width at attachment	-0.348	0.045
Stipule distance to max. width	-0.288	-0.050

absorbances intermediate between those of the parental species (Fig. 7). Absorbance data for the diagnostic wavelengths are given in Table 5.

*Electrophoretic observations.*—Preliminary electrophoretic observations on *B. australis*, *B. sphaerocarpa*, and the three F<sub>1</sub> hybrids show low levels of variation both within and between taxa. Eight of ten loci, *Aat*, *Idh*, *G3pdh*, *Mdh-1*, *Mdh-3*, *6Pgd*, *Skdh*, and *TPI-2*, were monomorphic with all individuals of all taxa expressing exactly the same alleles (one individual of *B. australis* did not express the *Mdh-1* allele). For *Mdh-2* all individuals were monomorphic for the *Mdh-2b* allele except one individual of *B. australis* and one individual *B. × bicolor* which also expressed *Mdh-2a*. For *Tpi-1* all individuals expressed *Tpi-1b* with most individuals being homozygous. However, one *B. × bushii* individual and one *B. × bicolor* individual were heterozygous *Tpi-1a* and *Tpi-1b*, while five *B. australis* individuals and two *B. × bicolor* individuals were heterozygous *Tpi-1b* and *Tpi-1c*.

*General observations.*—It is important to note that the spring flowering times of these species overlap by several weeks. *Baptisia bracteata* flowers first, followed by *B. australis* and *B. sphaerocarpa*, which flower at roughly the same time with some variation depending on the particular site. A significant number of *B. bracteata* individuals are still flowering when *B. australis* and *B. sphaerocarpa* come into flower. *Baptisia sphaerocarpa* tends to

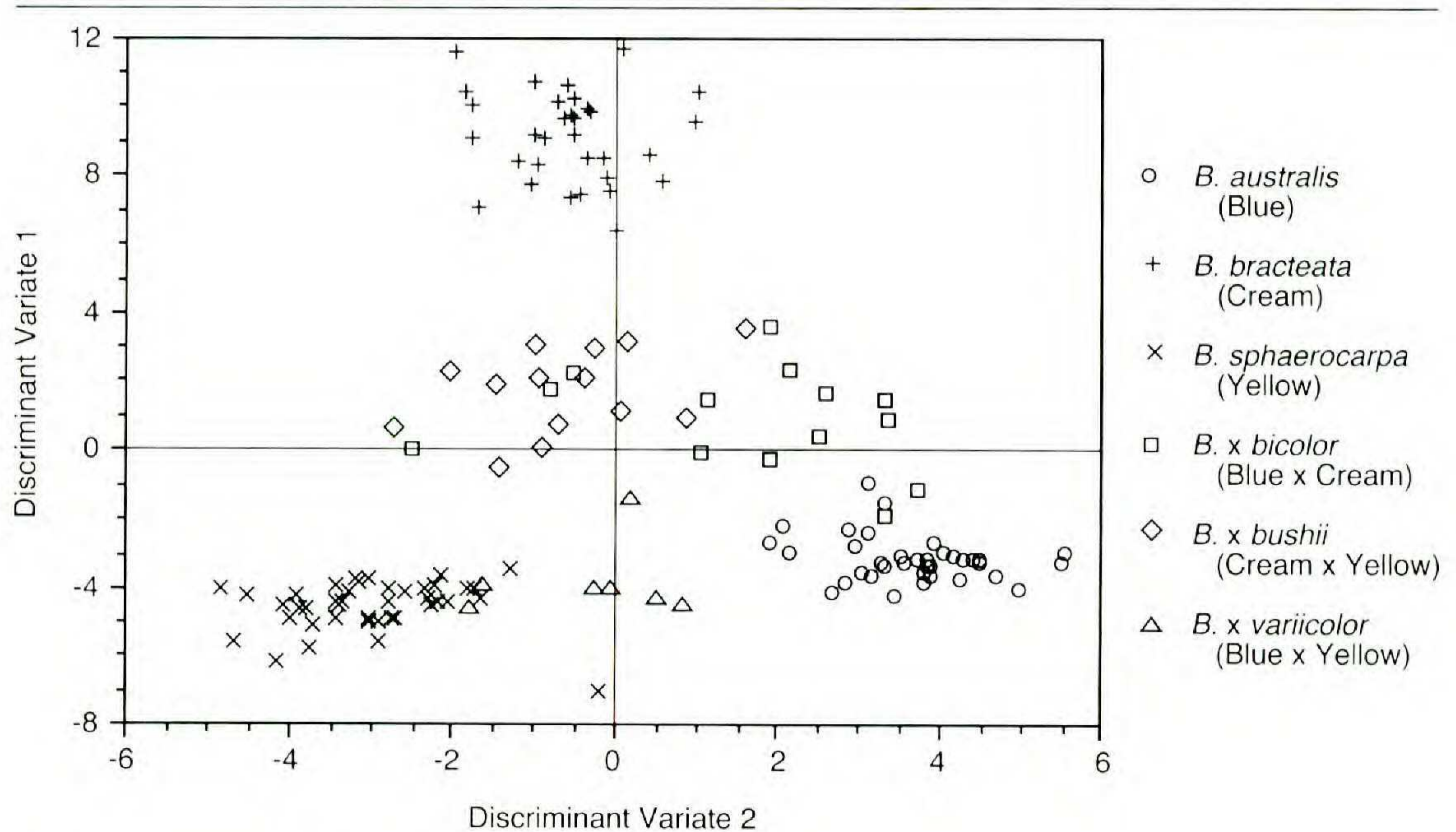


FIG. 6. Discriminant analysis.

flower somewhat longer, generally being the last of the species for which flowers can be observed. Hybrid flowering times in general are intermediate between those of the parent species. Over the course of field work during the 1996 flowering season, not only were the parental species simultaneously flowering, but also large bumble bees were observed indiscriminately visiting all three of the parents and hybrids (Fig. 3).

While several dozen hybrid individuals were observed, the number of individuals of the parental species are estimated in the hundreds or thousands. *Baptisia sphaerocarpa* in particular is quite abundant, being a vegetational dominant in overgrazed pastures. This species, which is apparently inedible to livestock, can have thousands of individuals in a single large field. At the sites containing more than one parental species, the expected hybrid(s) were always found, and the hybrids were always in much lower abundance than the parents. At the Fannin County site (FAN-A6) containing all six taxa, *B. australis* is fairly rare, while *B. sphaerocarpa* is the most common. *Baptisia variicolor* and *B. x bushii* are the two most common hybrids at that site. At the Grayson County site (PRE-A6) containing all six taxa, *B. sphaerocarpa* is rare, while *B. australis* is the most common. *Baptisia bicolor* and *B. x variicolor* are the more common hybrids at that site.

Soils differ significantly among sites; in the area studied, *B. australis* grows on Blackland clay soils, while *B. bracteata* and *B. sphaerocarpa* grow on sandy substrates. The hybrids involving *B. australis* occur either in the Preston Anticline in northern Grayson County where the soil types change rapidly allowing parental coexistence, or in western Fannin County in areas

TABLE 3. Linear discriminant functions.

Variable	<i>australis</i>	<i>bracteata</i>	<i>sphaerocarpa</i>	$\times$ <i>bushii</i>	$\times$ <i>bicolor</i>	$\times$ <i>variicolor</i>
Constant	-172.04	-202.93	-137.77	-155.08	-169.55	-141.85
Plant total height	0.30	0.04	0.02	-0.13	-0.01	-0.01
Plant veg. height	0.09	0.32	0.67	0.47	0.43	0.61
Raceme angle	-0.06	1.06	-0.09	0.36	0.37	-0.10
Flower length	4.10	3.05	3.61	3.24	3.95	3.65
Flower wing width	-0.58	0.30	0.62	0.24	-0.42	-0.10
Flower height	4.41	4.55	2.58	4.42	4.09	3.01
Flower banner width	3.74	2.31	2.85	2.87	3.56	3.52
Petiole length	-0.83	0.07	-0.86	-0.51	-0.79	-0.71
Pedicel length	-0.09	-3.38	0.01	-1.70	-0.61	0.11
Leaflet length	0.29	0.67	0.59	0.72	0.30	0.44
Leaflet width	1.88	1.03	2.06	1.71	1.74	1.89
L dist. to max. width	0.38	0.42	0.47	0.44	0.65	0.46
Stipule length	-1.40	-0.57	-1.39	-1.24	-1.44	-1.39
Stipule max. width	-0.50	0.90	-0.80	-0.89	-0.21	-1.13
S attachment width	4.88	5.85	2.88	6.77	6.11	4.53
S dist. to max. width	0.26	-4.69	-0.87	-1.71	-1.41	-0.07

of intermediate or mixed (a term used locally by early settlers) soil; plant communities closely parallel the geology of the area. *Baptisia bicolor* is known from only two sites within the study area, while the *B. australis*  $\times$  *B. sphaerocarpa* hybrid is known from three sites in the study area, but has also been reported from southern Oklahoma (Shirley Lusk, pers. comm.). Both *B. sphaerocarpa* and *B. bracteata* grow on sandy soils, and the plant distributions observed in the field suggest that the habitat separation between these two species is subtle. The hybrid of these two parental species (*B.  $\times$  bushii*) is by far the most common hybrid in terms of both number of individuals and number of sites at which they occur, which is to be expected since the parental species coexist more readily. The *B.  $\times$  bushii* sites range from grazed pastures (G36-CY and B36-CY) to native prairie (MEM-CY).

*Evidence for introgressive hybridization.*—Figure 8 shows that fruit lengths and widths of the two monospecific populations of *Baptisia australis* (GEO-B and PWR-B) were different from those of individuals of the same species from a site with *B. sphaerocarpa* and known hybrids (FAN-A6). Measurements of fruit length and width of *B. australis* from the hybrid site were intermediate between those of *B. australis* and *B. sphaerocarpa* (HAG-Y) from sites with only a single species. In contrast, fruits of *B. sphaerocarpa*

TABLE 4. Royal Horticultural Society Color comparisons.

Plant species	Color matches
<i>B. australis</i>	91A, 91B, 92A, 92C, 93A, 93B, 94A, 94B, 95A, 102A
<i>B. × variicolor</i>	61A, 17A, 91A, 92A, 92C, 94A, 94B, 9A, 14A
<i>B. sphaerocarpa</i>	9A
<i>B. × bushii</i>	9A, 9B, 14A, 14B
<i>B. bracteata</i>	10C, 10D
<i>B. × bicolor</i>	90A, 90B, 92A, 93A, 94A,

TABLE 5. Absorbance at diagnostic wavelengths.

Plant Type	Sample Size	422 nm		449 nm		540 nm	
		Ave	St. Err	Ave	St. Err	Ave	St. Err
<i>B. australis</i>	6	.18	.02	.20	.03	.85	.16
<i>B. × variicolor</i>	9	.45	.05	.43	.05	.88	.08
<i>B. sphaerocarpa</i>	6	.78	.05	.65	.04	.05	.01
<i>B. × bushii</i>	5	.37	.06	.33	.04	.03	.01
<i>B. bracteata</i>	6	.18	.03	.15	.02	.03	.01
<i>B. × bicolor</i>	4	.17	.02	.16	.02	.44	.10

from the hybrid site (FAN-A6) showed almost complete overlap in size with fruits of *B. sphaerocarpa* from a monospecific site (HAG-Y). These differences were confirmed by one-way analyses of variance that tested for differences in fruit length and fruit width as a function of group (the four groups: *B. australis* from monospecific sites, *B. australis* from hybrid site, *B. sphaerocarpa* from monospecific site, *B. sphaerocarpa* from hybrid site). Multiple comparisons tests (Fisher's) showed that there were no significant differences between the *B. sphaerocarpa* populations from the monospecific and hybrid sites, but all other pairwise comparisons were significantly different.

DISCUSSION

Two character plots, principal component analysis, and discriminant analysis all point to a conclusion similar to the one intuitively derived when these plants are observed in the field—that is, some plants are hybrids intermediate between the respective parental species. Principal component analysis also shows that within taxon morphological variation is less than between group variation. Based on raceme angle and flower color it is possible that backcrosses between F<sub>1</sub> hybrids and parental species (*B. × bushii* crossed with *B. bracteata* and *B. × bicolor* crossed with *B. australis*)

occur, possibly resulting in introgressive hybridization. At the Grayson County site containing all six species, several individuals are suggestive of this possibility. Two unusual *B. bracteata* individuals with racemes at a 45 degree angle to the ground were observed, as well as some *B. australis* individuals with multiple racemes and an atypically flexible stem structure.

A *B. australis* population (from a site also containing *B. sphaerocarpa* and known hybrids) with fruit length and width values intermediate between typical *B. australis* and *B. sphaerocarpa* is suggestive of introgression (Fig. 8). Evidence from additional characters, including genetic markers, is needed to confirm this hypothesis. In contrast, the lack of fruit differences between *B. sphaerocarpa* from the hybrid and monospecific sites suggests that genes from *B. astralis* are not moving into *B. sphaerocarpa*.

Discriminant analysis separates the groups well, but some individuals are ambiguously placed. Plants were originally grouped to taxon based on flower color. The discriminate analysis concluded that 6% of the individuals were not originally placed in the proper group (obvious stray points in Fig. 8). These plants may be backcrosses of F<sub>1</sub> hybrids parental species, products of introgression, or abnormal individuals. However, the fact that 94% of the individuals are correctly classified, the intermediate placement on the plot of the hybrids relative to the parents, and the intermediacy in non-quantitative characters (e.g. pubescence and bract morphology) leaves little doubt that this is a hybrid system.

Spectrophotometric data also support what the eye observes (Fig. 7). Pigment levels in both hybrids involving *B. bracteata* (*B. × bushii* and *B. × bicolor*) are intermediate (an average of the two parents) and in *B. × variicolor* flower color is additive (a sum of the two parents). The extensive flower color variation seen in *B. × variicolor* reflects to a certain extent the variation seen in *B. australis*, but clearly exceeds that level of variation. In some cases the keel is only yellow, while the banner is a deep brick-red or bluish-violet, while in other cases the flower is uniformly orangish or blue-violet-yellow. It is important to note that despite this dramatic variation in observable color, all *B. × variicolor* flowers exhibit nearly identical absorbance spectra (all have the same peaks with only minor differences in the actual absorbance). *Baptisia australis* also has variable flower pigmentation; in some flowers the keel is almost white, while the banner is a variable blue-violet, while in others the whole flower is a variable blue-violet. This variation in color saturation is also observed in the variable absorbance at the 540 nm peak. *Baptisia bracteata* and *B. sphaerocarpa* show little variation in flower coloration.

The spectral data suggest that compounds from both parents might be found in the hybrids and that the north central *Baptisia* hybrid swarm may be an excellent candidate for detailed chemotaxonomic investigation. In

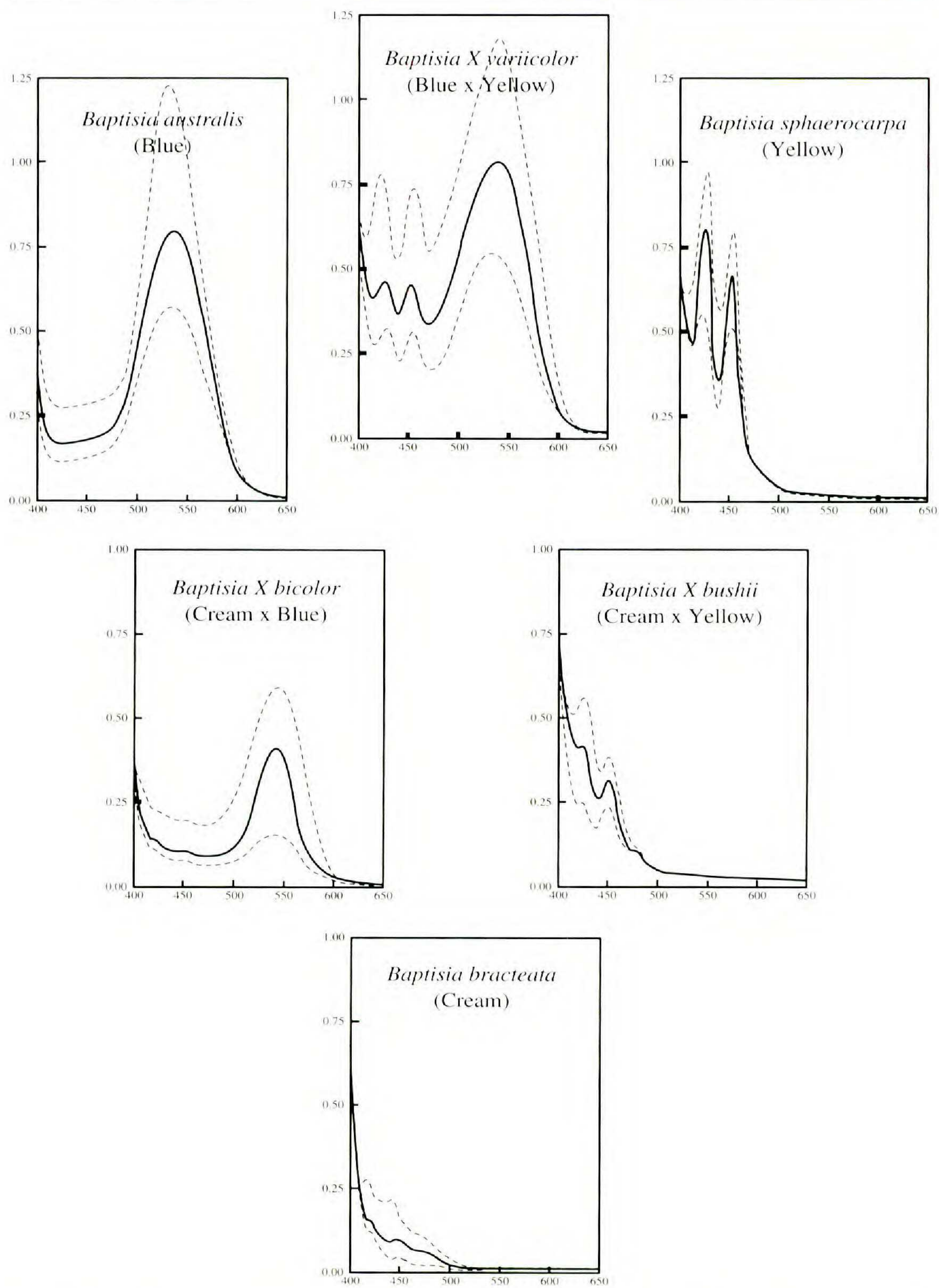


FIG. 7. Absorbance spectrum of flower extracts. Absorbance on the Y axis, wavelength (nm) on the X axis. Solid lines are mean absorbances (Table 5), dashed lines are the minimum and maximum absorbance spectra obtained.

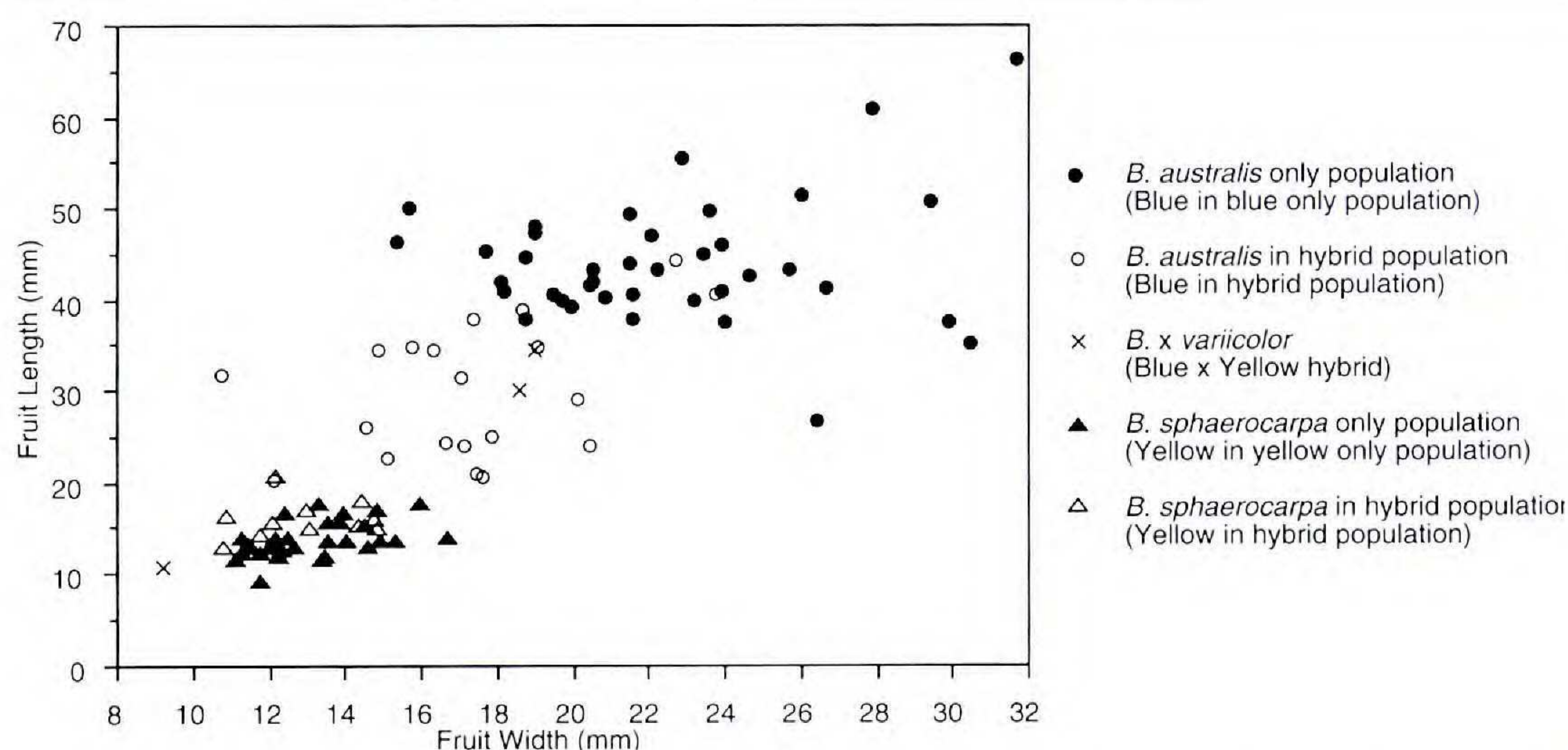


FIG. 8. Scatter diagram of fruit length and fruit width for *B. australis* from monospecific sites, *B. australis* from sites with *B. sphaerocarpa* and known hybrids, *B. variicolor*, *B. sphaerocarpa* from monospecific sites, *B. sphaerocarpa* from sites with *B. australis* and known hybrids. Blue-flowered individuals (*B. australis*) from the hybrid population are intermediate in fruit shape between *B. sphaerocarpa* and *B. australis* from sites with only a single species; this is suggestive of introgression.

their detailed study of *Baptisia* flavonoid chemistry, Markham et al. (1970), and later Dement and Mabry (1975), divide *Baptisia* into subgroupings based on the distribution of flavonoid pigments throughout the genus, placing *B. sphaerocarpa* and *B. australis* in separate subgroups within the genus because of large (relative to other members of the genus) chemical differences. In contrast to this, our preliminary electrophoretic data (eight of 10 loci monomorphic and identical) point to a close relationship between *B. sphaerocarpa* and *B. australis*. This similarity is consistent with the apparent ease with which hybrids occur. Overall, hybridization, morphological similarity, and electrophoretic data all suggest a close relationship between the species. These findings possibly suggest that the chemotaxonomic subgroupings may not be reflective of phylogeny. In either case this hybrid complex warrants further investigation.

#### TAXONOMIC TREATMENT

Herbaceous perennials with woody crown and tough roots; leaves very short-petioled, palmately compound with 3 leaflets (or uppermost with only 1 or 2); stipules large to small, persistent or falling early; flowers terminal, large, solitary or in erect to horizontal or drooping racemes or solitary in the leaf axils; corollas variously colored; stamens 10, separate; fruit rounded or subcylindric, becoming woody, often with a beak. The plants are avoided by grazing animals; some species are reportedly toxic to livestock.

*Baptisia nuttalliana* Small occurs in north central Texas to the south of the study area but is not involved in the hybrid complex discussed here. It can be distinguished by the yellow flowers occurring singly in the upper leaf axils (at least some) or in very short terminal racemes with 1–4 flowers. The only other Texas *Baptisia* species is *B. alba* (L.) Ventenat var. *macrophylla* (Larisey) Isely [= *B. leucantha* Torr. & Gray], known only from sandy soil in east and southeast Texas. It is distinguished by its erect inflorescences with white flowers subtended by early deciduous bracts.

KEY TO NORTH CENTRAL TEXAS *BAPTISIA* PARENTAL AND HYBRID TAXA

1. Petals cream; inflorescence held distinctly below the level of the leaves; vegetative structures densely pubescent ..... *B. bracteata*
1. Petals blue-violet to yellow, orange, or brick-red, but not cream; inflorescence at least partially erect and held above the level of the leaves or solitary in the upper leaf axils; vegetative structures glabrous to moderately pubescent.
  2. Petals blue-violet (keel sometimes whitish); stems at base thick (>5 mm diam.); plants usually growing individually; inflorescences one to a few per plant.
    3. Petals intensely to moderately blue-violet; inflorescence vertical (strictly erect); plant glabrous and glaucous ..... *B. australis*
    3. Petals moderately to pale blue-violet; inflorescence angled about 45°; plant slightly pubescent, not glaucous ..... *B. × bicolor*
  2. Petals yellow or multicolored; stems at base thin (<5 mm diam.); plants usually growing in dense clusters; inflorescences usually numerous per plant.
    4. Petals yellow, all ± the same color; foliage glabrous to moderately pubescent.
      5. Petals vividly yellow; raceme nearly vertical (strictly erect); vegetative portions of the plant glabrous ..... *B. sphaerocarpa*
      5. Petals pale yellow; raceme angled about 45°; vegetative portions of the plant moderately pubescent ..... *B. × bushii*
    4. Petals brick-red and yellow to orangish to blue-violet and yellow, different petals of the same flower often of different colors; foliage glabrous ..... *B. × variicolor*

***Baptisia australis* (L.) R. Br. ex W.T. Aiton var. *minor* (Lehm.) Fern., Rhodora 39:312. 1937.**

*Baptisia minor* Lehm., Ind. Seimin. Hort. Hamb. 16. 1827.

*Baptisia minor* var. *aberrans* Larisey, Ann. Missouri Bot. Gard. 27:206. 1940.

*Baptisia texana* Buckl., Proc. Acad. Nat. Sci. Philadelphia 13:452. 1861.

*Baptisia versicolor* Loddiges, Bot. Cab. 12: pl. 1144. 1826.

*Baptisia vespertina* Small ex Rydb., Fl. Prairies & Plains Central N. Amer. 456. 1932.

Plants usually growing individually, tall (usually 46–74 cm), of strictly erect habit, with a single, thick (>5 mm diam.), glaucous stem rising from the ground several cm before branching; branches generally few and rigid; leaves small (center leaflet 18–34 mm long, average 25 mm) and glabrous; stipules small (4–11(–22) mm long); flowers borne on a usually single,

rigidly erect raceme; floral bracts early deciduous; pedicels 5–15 mm long; corollas large (27–36 mm long), blue-violet of variable intensity; fruit usually much longer than wide, 30–60 mm long, 20–30 mm wide, with a distinct persistent beak that is noticeably widened at base, black at maturity, glabrous. Clay soil, prairies, pastures; Fannin and Grayson cos. in Red River drainage, also Collin Co., also Dallas Co. (Mahler 1988); otherwise only known in Texas from the Panhandle and Titus Co. to the east. Flowering Apr.

***Baptisia* × *bicolor*** Greenm. & Larisey (*B. australis* × *B. bracteata*), Amer. J. Bot. 26:539. 1939.

Plants usually growing individually, intermediate to both parents in numerous characters, of medium height (30–60 cm tall), generally branching at or near the ground; stems and foliage moderately pubescent; leaves and stipules of moderate length (center leaflet 27–51 mm long; stipules 4–10 mm long); flowers borne on 1–several racemes angled about 45° above the ground; floral bracts early deciduous; pedicels 10–18 mm long; corollas large (27–33 mm long), light to medium blue-violet. Prairies, pastures, soils often intermediate between clay and sand or soil types in close proximity; Red River drainage, Fannin and Grayson cos. Flowering Apr.

***Baptisia bracteata* Muhl. ex Ell. var. *leucophaea* (Nutt.) Kartesz & Gandhi**, Phytologia 71:276. 1991.

*Baptisia bracteata* var. *glabrescens* (Larisey) Isely, Brittonia 30:470. 1978.

*Baptisia leucophaea* Nutt., Gen. N. Amer. Pl. 1:282. 1818.

*Baptisia leucophaea* var. *glabrescens* Larisey, Ann. Missouri Bot. Gard. 27:161. 1940.

*Lasinia bracteata* Raf., New Fl. N. Amer. 2:84. 1910.

Plants usually growing individually, low (21–36 cm tall), often wider than tall, branching at or near the ground, with all vegetative parts densely pubescent; leaves and stipules large (center leaflet 30–60 mm long; stipules 10–40 mm long); flowers on reclining or hanging racemes; floral bracts persistent and large (10–30 mm long); pedicels long (15–35 mm long); corollas large (25–38 mm long); fruit (2–)3–4(–5) cm long, 1.5–2.5 cm wide, tapering to a slender beak (wide at base), black, pubescent or rarely later glabrate. Sandy open woods, prairies, pastures, and roadsides; East Texas west to East Cross Timbers. Flowering late Mar–Apr.

***Baptisia* × *bushii*** Small (*B. bracteata* × *B. sphaerocarpa*), Fl. SE U.S. 600. 1903.

*Baptisia* × *intermedia* Larisey, Ann. Missouri Bot. Gard. 27:165. 1940.

*Baptisia* × *stricta* Larisey, Ann. Missouri Bot. Gard. 27:166. 1940.

Plants usually growing individually, intermediate to both parents in numerous characters, low (26–36 cm tall), branching at or near the ground; stems and foliage moderately pubescent; leaves and stipules moderate to

large (center leaflet 42–60 mm long; stipules 7–34 mm long); flowers on several racemes angled about 45° above the ground; floral bracts early deciduous; pedicels 7–15 mm long; corollas medium-sized (22–29 mm long), light to medium yellow. Usually on sandy soil; Red River drainage, Fannin and Grayson cos. Flowering Apr.

**Baptisia sphaerocarpa** Nutt., J. Acad. Nat. Sci. Philadelphia 7:97. 1834.

*Baptisia viridis* Larisey, Ann. Missouri Bot. Gard. 27:196. 1940.

Plants often growing in a dense cluster with a number of apparently identical individuals in close proximity, suggesting vegetative spread, tall (40–70 cm) and of generally erect habit, with 1-many small, flexible, glabrous stems, branching close to the base and often; leaves large (center leaflet 36–67 mm long), the upper leaves often reduced to 1–2 leaflets; stipules small (2–5(–6.1) mm long) to absent; flowers borne on 1-many flexible vertical racemes; floral bracts early deciduous; pedicels short (1–7 mm long); corollas small (17–25 mm long), intensely yellow; fruit subspheroid, 1.4–1.8 mm in diam., usually light brown [Isely (1990) also reports black], glabrous; in north central Texas the small, nearly round, light brown fruits immediately distinguish *B. sphaerocarpa* from the other two parental species with larger, elongate, black fruits. Sandy or silty clay; east Texas west to western Blackland Prairie (Grayson Co.). Flowering Apr–May.

**Baptisia × variicolor** Kosnik, Diggs, Redshaw, & Lipscomb, nothosp. nov. (Figs. 1 & 3)

Inter parentes *B. australem* (L.) R. Br. ex W.T. Aiton et *B. sphaerocarpan* Nutt. habitu intermedia, florum colore e lateritio, flavo vel aurantio violascenti vel lutescenti recognitur. (*B. australis* × *B. sphaerocarpa*).

Plants often growing in a dense cluster with a number of apparently identical individuals in close proximity, suggesting vegetative spread, intermediate to both parents in numerous characters, but vegetatively often more similar to *B. sphaerocarpa* than *B. australis*, tall (40–76 cm) and of generally erect habit, with 1-many small, flexible, glabrous stems, branching at or near the base and often; leaves medium-sized (center leaflet 30–50 mm long); stipules small (3–15 mm long) to absent; flowers borne on 1-many flexible vertical racemes; floral bracts early deciduous; corollas of intermediate size (22–27 mm long), brick-red and yellow to orangish to bluish-violet and yellow. Prairies, pastures, soils often intermediate between clay and sand or soil types in close proximity; Red River drainage, Fannin and Grayson cos. Flowering Apr–May. Named for the extremely variable flower color. The epithet is spelled *variicolor*, so that the first “i” is part of the root, *vari*.

TYPE: U.S.A. TEXAS: Fannin Co.: 5 mi E of Whitewright, in Ely Community, on Farm Road 898, unplowed native prairie remnant, 33° 31' N, 96° 20' W, elevation 253 m, 25 Apr 1996, Kosnik, Diggs, & Wolford 108 (HOLOTYPE: BRIT; ISOTYPES: BRIT, TEX, US).

#### CONCLUSIONS

Morphological and spectrophotometric data are consistent with the hypothesis that visually intermediate individuals from Grayson and Fannin counties, Texas represent hybrids between the three locally sympatric species. At two study sites there are previously undocumented hybrid swarms of all three parental species and all three  $F_1$  hybrids. Variation suggestive of backcrossing between parents and hybrids and introgressive hybridization was also observed. The range of the hybrids, *B. × bushii* and *B. × bicolor*, has been extended to include populations from north central Texas and a new hybrid, *B. × variicolor* is described. This hybrid is morphologically intermediate and additive in terms of flower color between the parents, but is often vegetatively more similar to *B. sphaerocarpa*; the variable brick-red and yellow to orangish to bluish-violet and yellow coloration of the flower is distinctive. The coloration and absorbance data suggest a summation of the parental pigment pathways, with both parental pigments being expressed in *B. × variicolor*. In the study area this hybrid only occurs where different soils are in close proximity or where soils are of an intermediate or mixed nature.

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#### REFERENCES

- ALSTON, R.E. and TURNER, B.L. 1962. New techniques in analysis of complex natural hybridization. *Proc. Natl. Acad. U.S.A.* 48:130–137.  
———. 1963. Natural hybridization among four species of *Baptisia* (Leguminosae). *Amer. J. Bot.* 50:159–173.  
ANDERSON, E. 1949. *Introgressive hybridization*. John Wiley & Sons, New York.

- CORRELL, D.S. and M.C. JOHNSTON. 1970. Manual of the vascular plants of Texas. Texas Research Foundation, Renner.
- DEMENT, W.A. and T.J. MABRY. 1975. Biological implications of flavonoid chemistry in *Baptisia* and *Thermopsis*. *Biochem. Syst. Ecol.* 3:91–94.
- ENGELMANN, G. 1878. *Baptisia sulphurea*, n. sp. *Bot. Gaz. (Crawfordsville)* 3:65.
- HARPER, R.M. 1938. A simple-leaved *Baptisia* from the coast prairies of Louisiana, and a supposed hybrid. *Torreyia* 38:121–124.
- HITCHCOCK, A.S. 1894. A hybrid *Baptisia*. *Bot. Gaz. (Crawfordsville)* 19:42.
- ISELY, D. 1990. Leguminosae (Fabaceae). *Vascular flora of the southeastern United States* 3(2):1–258.
- KARTESZ, J.T. 1994. A synonymized checklist of the vascular flora of the United States, Canada, and Greenland. 2nd edition. Timber Press, Portland, Oregon.
- KOSNIK, M.A. 1996. Natural hybridization among three sympatric *Baptisia* (Fabaceae) species in north central Texas. Honors thesis. Austin College, Sherman, Texas.
- LARISEY, M.M. 1940a. A monograph of the genus *Baptisia*. *Ann. Missouri Bot. Gard.* 27:119–258.
- . 1940b. Analysis of a hybrid complex between *Baptisia leucantha* and *Baptisia viridis* in Texas. *Amer. J. Bot.* 27:624–628.
- MAHLER, W.F. 1988. Shinnery's manual of the north central Texas flora. *Sida, Bot. Misc.* No. 3.
- MARKHAM, K.R., T.J. MABRY, and W.T. SWIFT, JR. 1970. Distribution of flavonoids in the genus *Baptisia* (Leguminosae). *Phytochemistry* 9:2359–2364.
- MINITAB 10.5Xtra. 1995. Statistical software by Minitab, Inc. State College, PA.
- PIMENTEL, R.A. 1995. BioStat II: A Multivariate Statistical Toolbox. Version 3.5. Statistical software by Sigma Soft. San Luis Obispo, CA.
- ROYAL HORTICULTURAL SOCIETY COLOUR CHART. 1966. Royal Horticultural Society, London.
- SOLTIS, D.E., C.H. HAUFER, D.C. DARROW, and G.J. GASTONY. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *Amer. Fern J.* 73:9–27.
- and P.S. SOLTIS. 1987. Breeding system of the fern *Dryopteris expansa*: evidence for mixed-mating. *Amer. J. Bot.* 74:504–509.
- TURNER, B.L. 1959. The legumes of Texas. University of Texas Press, Austin.
- and R.E. ALSTON. 1959. Segregation and recombination of chemical constituents in a hybrid swarm of *Baptisia laevicaulis* × *B. viridis* and their taxonomic implications. *Amer. J. Bot.* 46:678–686.